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ACQUISITION OF ACOUSTIC STARTLE SHOWS A DOSE-RESPONSE TO SERUM FREE T_4

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Abstract—Methimazole, administered to rats in drinking water (0.1 and 0.05%) from embryonic day 17 to postnatal day 10, caused a dose-dependent decrease in serum Free T_4 and an accompanying dose-dependent delay in acquisition of acoustic startle reflex. In conjunction with other studies showing the specific dependence of acoustic startle development on thyroid hormone, this study suggests that the acoustic startle system may be a useful model for determining thyroid hormone requirements for normal neurologic development.

Key words: Acoustic startle reflex, T_4, Methimazole, Thyroid hormone.

It is well known that severe perinatal hypothyroidism impairs development of the nervous system[10] with consequences ranging from mild to profound and permanent mental retardation.[9,11,13] Deafness, or milder forms of hearing loss are other known consequences.[21] Though it is clear that lack of thyroid hormone during development can cause these effects, it is not clear how much hormone is necessary for normal development.

Many different drugs and chemicals have antithyroid effects,[20] and are thus a potentially serious hazard to the developing fetus. Also, genetic disorders such as Down's Syndrome and Penred's Syndrome have associated thyroid disturbances.[12,14,6] To evaluate the impact of such environmental or genetic factors on neurologic development it is essential to establish a quantitative relationship between thyroid hormone and development of the nervous system.

In establishing this quantitative relationship one must be able to monitor the development of a neurologic system that is dependent on thyroid hormone, but which is not affected secondarily by other consequences of hypothyroidism. Further, it is necessary to demonstrate that the degree of developmental disruption correlates with the degree of hypothyroidism, i.e. a dose-response relationship.

Previous studies have shown that perinatal hypothyroidism— induced in rodents with antithyroid compounds—retards or inhibits acquisition of the acoustic startle response,[1,6] and it has been shown that this response is accelerated by administration of thyroxine to neonatal animals.[17,24] Thus, it is reasonable to hypothesize that acquisition of acoustic startle response is dose-responsive to concentrations of thryoid hormone. Since our primary interest is in the minimal amount of hormone needed for normal development, we have tested this hypothesis in the hypothyroid region of the dose-response curve. In this report we show that methimazole causes a dose-dependent decrease in serum concentrations of Free T_4, and that this same treatment schedule causes a dose-dependent delay in acquisition of acoustic startle. These data support the hypothesis that acquisition of acoustic startle response displays a dose-response to serum concentrations of thyroxine.

EXPERIMENTAL PROCEDURES

Animals

Sprague–Dawley derived rats were made hypothyroid during the perinatal period by administering methimazole in the drinking water of their mothers from embryonic day 17 (day 1 = sperm positive) until postnatal day 10 (PN10; PN1 = day of birth). This treatment period extends from the beginning of thyroid hormone production,[7,15] until a stage in rat development approximately comparable to that of humans at birth.[5]

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Two experimental groups were established, with each containing: control, low dose (0.05% methimazole in water), and high dose (0.1%) treatments. Group 1 comprised 6 control dams, 4 low dose dams, and 3 high dose dams with their respective litters. Group 1 was tested daily from PN10 until each animal showed a positive response to the acoustic startle. The number of neonates tested for acoustic startle were: control = 47, low dose = 32, high dose = 23.

Group 2 was killed on PN10 for determining thyroid hormone concentrations. The number of samples analyzed for hormone concentrations were: control = 19, low dose = 21, high dose = 17. Group 2 comprised 3 dams each for control, low dose, and high dose treatments.

Acoustic startle

Presence of the acoustic startle response was determined daily from PN10 until its appearance. Rat pups were placed individually on a flat surface and exposed to a sudden impact noise, produced by dropping a steel rod from a height of 40 cm onto a stainless steel bowl suspended 30 cm above the animal. A positive response was recorded when an animal, standing on all four feet, made a distinctly visible extension of its limbs in response to the noise stimulus.

Using a Bruel and Kjaer Impulse Precision Sound Level Meter (Type 2209) with a 1/3 octave filter set (Type 1616), it was determined that the noise stimulus had an intensity of 134 ± 1 dB with a peak frequency of 1 kHz (peak range = 1–8 kHz). Acoustic intensity reference = 2×10^{-4} dyne/cm^2. Background noise intensity was 58 dB.

Thyroid hormone

For measurements of thyroid hormone (Free T_4) blood was obtained by cardiac puncture of ether-anesthetized rat pups on PN10. Whole blood samples were refrigerated overnight. Serum was separated from plasma by centrifugation, removed by syringe, and immediately frozen. Thyroxin concentrations were determined using a standard ^{125}I radioimmunoassay.

Materials

Methimazole (M 8506) was purchased from Sigma Chemical Co. (St Louis, MO, U.S.A.). ^{125}I Radioimmunoassay kits (CA-535) for thyroxine were purchased from Travenol-Genentech Diagnostics.

RESULTS

Perinatal methimazole caused a stastically significant dose-dependent decrease in serum Free T_4 (Table 1), and a significant dose-dependent delay in development of the acoustic startle response (Table 2). Statistical significance of differences between mean values was determined using Student's *t*-test.

Figure 1 graphically shows the linear dose-response relationship obtained by plotting mean Free T_4 concentrations (log scale) and mean values for acquisition of acoustic startle response.

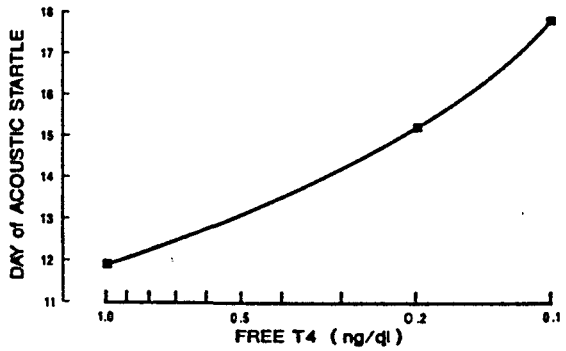

Fig. 1. Correlation between acquisition of acoustic startle response and serum Free T_4 concentrations (log scale) after perinatal methimazole treatment. Mean values (from Tables 1 and 2) are plotted for respective groups.

Table 1. Free T_4 concentrations ($\bar{X} \pm$ S.E.) on postnatal day 10 for rats treated with methimazole from embryonic day 17

	Serum Free T_4 (ng/100 ml)
Control	1.0 ± 0.05
Methimazole (0.05%)	0.2 ± 0.02*
Methimazole (0.1%)	0.1 ± 0.01*†

* Significantly different from control, $P<0.01$.
† Significantly different from 0.05% methimazole, $P<0.025$.

Table 2. Acquisition of acoustic startle response in rats treated with methimazole from embryonic day 17 to postnatal day 10

	Day of acquisition ($\bar{X} \pm$ S.E.)
Control	11.9 ± 0.1
Methimazole (0.05%)	15.2 ± 0.2*
Methimazole (0.1%)	17.8 ± 0.4*†

* Significantly different from control, $P<0.001$.
† Significantly different from 0.05% methimazole, $P<0.001$.

Free T_4 concentrations are expressed as nanograms (ng) of thyroxine per 100 ml (1 dl) of serum.

DISCUSSION

Previous studies have shown that thyroxine supplementation after perinatal administration of antithyroid compounds prevents the delay in acquisition of acoustic startle, and mitigates the abnormalities in cochlear development.[3,22] Thus, it is reasonable to assume in our study that the differential delay in acoustic startle development, after two different doses of methimazole, is causally related to the dose-dependent effect of methimazole on Free T_4 concentrations. As far as we know, this is the first time that a dose-response relationship has been demonstrated from serum thyroxine concentrations and development of a neurobehavioral response. The demonstration of this phenomenon becomes more exciting when one considers that this response is highly dependent upon thyroid hormone for normal development, but does not appear to be affected secondarily by consequences of antithyroid treatment such as undernutrition and poor growth.[3,18,19]

The acoustic startle pathway has been well characterized in adult rats.[2] Though at this time we cannot say what part(s) of this pathway (e.g. middle ear, inner ear, cochlear nucleus, etc.) is affected in a dose-dependent manner, it is reasonable to hypothesize—in light of the studies which demonstrate the responsiveness of cochlear development and synaptogenesis to thyroxine[3,4,19,20]—that cochlear development will, also, show a dose-response to thyroid hormone. Thus, while development of acoustic startle reflex may be a useful neurobehavioral model for establishing a critical concentration of the hormone necessary for normal neurologic development, studies of cochlear development may provide an interesting morphologic correlate.

Based on the dose-response relationship in Fig. 1 we have estimated that a 50% decrease in Free T_4 would have clearly demonstrable effects in our present experimental paradigm. In making this estimate it is assumed that an average delay of 1 day in acquisition of acoustic startle would be both necesary and sufficient for detection (this assumption relies on the statistical generalization that a mean difference of >4 S.E.s will probably be statistically significant, and is based on the S.E. of the low dose mean response).

However, with our present experimental protocol, antithyroid treatment was stopped on PN10, whereas acoustic startle does not develop normally until PN12. Because methimazole has a short biological half-life—about 5 hr in adult rodents[7]—the treated groups had probably begun to regain normal thyroid function and normal Free T_4 levels by the time they developed the acoustic startle response (on average, days 15 or 18). Thus, we predict that with continuous treatment an even lesser degree of hypothyroidism, produced by lower doses of methimazole, will cause a significant neurobehavioral deficit. Further research will be needed to determine how much of a change in perinatal thyroid function may have detremental effects on the development of structure and function in the auditory system.

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